

WHAT IS CLAIMED IS:

✓ 1. ICAM-1, or a functional derivative thereof, substantially free of natural contaminants.

5 2. The ICAM-1 of claim 1, wherein said ICAM-1 is additionally capable of binding to a molecule present on the surface of a lymphocyte.

3. The ICAM-1 molecule of claim 2, wherein said molecule additionally contains at least one polypeptide selected from the group consisting of:

10 (a) -V-T-C-S-T-S-C-D-Q-P-K;
(b) -X-G-S-V-L-V-T-C-S-T-S-C-D-Q-P-K;
(c) -L-L-G-I-E-T-P-L;
(d) -F-L-T-V-Y-X-T;
(e) -V-E-L-A-P-L-P;
15 (f) -E-L-D-L-R-P-Q-G-L-E-L-F-E;
(g) -L-N-P-T-V-T-Y-G-X-D-S-F-S-A-K;
(h) -S-F-P-A-P-N-V;
(i) -L-R-G-E-K-E-L;
(j) -R-G-E-K-E-L-K-R-E-P;
20 (k) -L-R-G-E-K-E-L-K-R-E-P-A-V-G-E-P-A-E;
(l) -P-R-G-G-S;
(m) -P-G-N-N-R-K;
(n) -Q-E-D-S-Q-P-M;
(o) -T-P-E-R-V-E-L-A-P-L-P-S;
25 (p) -R-R-D-H-H-G-A-N-F-S; and
(q) -D-L-R-P-Q-G-L-E.

4. A functional derivative of the ICAM-1 of claim 2, which functional derivative is capable of binding to a molecule present on the surface of a lymphocyte, wherein said functional derivative is a fragment, variant, analog or chemical derivative of ICAM-1.

30

5. The functional derivative of claim 4, which contains the following amino acid(s) at the indicated position: S3VS, V4, R13, D26PK, Q27, E34, G46NN, K50V, Q58EDS, D60S, D71GQS, Q73, Y83, E90, N103, A115N, or N175TSA.

6. The functional derivative of claim 4 which contains:

- (1) domains 1, 2, 3, 4 and 5 of ICAM-1;
- (2) domains 1, 2, 3 and 4 of ICAM-1;
- (3) domains 1, 2 and 3 of ICAM-1;
- (4) domains 1 and 2 of ICAM-1; or
- (5) domain 1 of ICAM-1.

7. A recombinant DNA molecule capable of expressing ICAM-1 or a functional derivative thereof.

8. The DNA molecule of claim 7, wherein said DNA molecule is capable of encoding at least one polypeptide selected from the group consisting of:

- (a) -V-T-C-S-T-S-C-D-Q-P-K;
- (b) -X-G-S-V-L-V-T-C-S-T-S-C-D-Q-P-K;
- (c) -L-L-G-I-E-T-P-L;
- (d) -F-L-T-V-Y-X-T;
- (e) -V-E-L-A-P-L-P;
- (f) -E-L-D-L-R-P-Q-G-L-E-L-F-E;
- (g) -L-N-P-T-V-T-Y-G-X-D-S-F-S-A-K;
- (h) -S-F-P-A-P-N-V;
- (i) -L-R-G-E-K-E-L;
- (j) -R-G-E-K-E-L-K-R-E-P;
- (k) -L-R-G-E-K-E-L-K-R-E-P-A-V-G-E-P-A-E;
- (l) -P-R-G-G-S;
- (m) -P-G-N-N-R-K;
- (n) -Q-E-D-S-Q-P-M;
- (o) -T-P-E-R-V-E-L-A-P-L-P-S;
- (p) -R-R-D-H-H-G-A-N-F-S; and
- (q) -D-L-R-P-Q-G-L-E.

9. A method for recovering ICAM-1 in substantially pure form which comprises the steps:

- (a) solubilizing ICAM-1 from the membranes of cells expressing ICAM-1, to form a solubilized ICAM-1 preparation,
- (b) introducing said solubilized ICAM-1 preparation to an affinity matrix, said matrix containing immobilized antibody capable of binding to ICAM-1,

(c) permitting said ICAM-1 to bind to said antibody of said affinity matrix,

(d) removing from said matrix any compound incapable of binding to said antibody and

5 (e) recovering said ICAM-1 in substantially pure form by eluting said ICAM-1 from said matrix.

10. The method of claim 9 which additionally comprises the steps:

(f) ~~purifying said recovered ICAM-1~~ of step (e) by preparative gel electrophoresis, and

10 (g) eluting said recovered ICAM-1 from a gel employed in step (f).

11. The ICAM-1 produced by the method of any one of claims 9-10.

✓ 12. The antibody R6-5-D6, or a fragment thereof, wherein said antibody or said fragment is capable of binding to ICAM-1.

15 13. The antibody of claim 12, in labeled form.

14. A hybridoma cell which is capable of producing the monoclonal antibody of claim 12, said cell being ATCC HB 9580.

✓ 15. A method of identifying a non-immunoglobulin antagonist of intercellular adhesion which comprises:

20 (a) incubating a non-immunoglobulin agent capable of being an antagonist of intercellular adhesion with a lymphocyte preparation, said lymphocyte preparation containing a plurality of cells capable of aggregating,

25 (b) examining said lymphocyte preparation to determine whether the presence of said agent inhibits the aggregation of said cells of said lymphocyte preparation; wherein inhibition of said aggregation identifies said agent as an antagonist of intercellular adhesion.

16. A method for treating inflammation resulting from a response of the specific defense system in a mammalian subject which comprises providing to a subject in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress said inflammation; wherein said anti-inflammatory agent is selected from the group consisting of: an antibody capable of binding to ICAM-1; a fragment of an antibody, said fragment being capable of binding to ICAM-1; ICAM-1; a functional derivative of ICAM-1; and a non-immunoglobulin antagonist of ICAM-1.

17. The method of claim 16, wherein said non-immunoglobulin antagonist of ICAM-1 is ~~a non-immunoglobulin~~ antagonist of ICAM-1 other than LFA-1.

18. The method of claim 16, wherein said functional derivative of ICAM-1 is a fragment of ICAM-1.

19. The method of claim 16, wherein said functional derivative of ICAM-1 contains the following amino acid(s) at the indicated positions: S3VS, V4, R13, D26PK, Q27, E34, G46NN, K50V, Q58EDS, D60S, D71GQS, Q73, Y83, E90, N103, A115N, or N175TSA.

20. The method of claim 16, wherein said functional derivative contains:

- (1) domains 1, 2, 3, 4 and 5 of ICAM-1;
- (2) domains 1, 2, 3 and 4 of ICAM-1;
- (3) domains 1, 2 and 3 of ICAM-1;
- (4) domains 1 and 2 of ICAM-1; or
- (5) domain 1 of ICAM-1.

21. The method of claim 16, wherein said ICAM-1 or said functional derivative of ICAM-1 is a soluble protein.

22. The method of claim 21, wherein said soluble protein lacks the transmembrane domain of ICAM-1.

23. The method of claim 21, wherein said soluble protein lacks the cytoplasmic domain of ICAM-1.

5 24. The method of claim 16, wherein said anti-inflammatory agent is an antibody capable of binding to ICAM-1, or a fragment of said antibody, said fragment being capable of binding to ICAM-1.

25. The method of claim 24, wherein said antibody is a monoclonal antibody.

26. The method of claim 25, wherein said monoclonal antibody is the monoclonal antibody R6-5-D6.

10 27. The method of claim 16, wherein said inflammation is a delayed type hypersensitivity reaction.

28. The method of claim 16, wherein said inflammation is a symptom of psoriasis.

15 29. The method of claim 16, wherein said inflammation is a symptom of an autoimmune disease.

30. The method of claim 29, wherein said autoimmune disease is selected from the group consisting of Reynaud's syndrome, autoimmune thyroiditis, EAE, multiple sclerosis, rheumatoid arthritis and lupus erythematosus.

20 31. The method of claim 16, wherein said inflammation is in response to organ transplant rejection.

32. The method of claim 31, wherein said organ transplant is a kidney transplant.

33. The method of claim 16, wherein said inflammation is in response to tissue graft rejection.

34. The method of claims 16 which additionally comprises the administration of an agent selected from the group consisting of: an antibody capable of binding to LFA-1; a functional derivative of an antibody, said functional derivative being capable of binding to LFA-1; and a non-immunoglobulin antagonist of LFA-1.

35. A method for treating inflammation resulting from a response of the non-specific defense system in a mammalian subject which comprises providing to a subject in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress said inflammation; wherein said anti-inflammatory agent is a functional derivative of ICAM-1; wherein said functional derivative of ICAM-1 contains the following amino acid(s) at the indicated positions: S3VS, V4, R13, D26PK, Q27, E34, G46NN, K50V, Q58EDS, D60S, D71GQS, Q73, Y83, E90, N103, A115N, or N175TSA.

36. A method for treating inflammation resulting from a response of the non-specific defense system in a mammalian subject which comprises providing to a subject in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress said inflammation; wherein said anti-inflammatory agent is a functional derivative of ICAM-1; wherein said functional derivative contains:

- (1) domains 1, 2, 3, 4 and 5 of ICAM-1;
- (2) domains 1, 2, 3 and 4 of ICAM-1;
- (3) domains 1, 2 and 3 of ICAM-1;
- (4) domains 1 and 2 of ICAM-1; or
- (5) domain 1 of ICAM-1.

37. A method for treating inflammation resulting from a response of the non-specific defense system in a mammalian subject which comprises providing to a subject in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress said inflammation; wherein

said anti-inflammatory agent is a functional derivative of ICAM-1; wherein said functional derivative of ICAM-1 is a soluble protein.

38. The method of claim 37, wherein said soluble protein lacks the transmembrane domain of ICAM-1.

5 39. The method of claim 37, wherein said soluble protein lacks the cytoplasmic domain of ~~ICAM-1~~.

40. The method of any of claims 16, or 35-37, which additionally comprises the administration of an immunosuppressive drug.

10 41. The method of claim 40, wherein said immunosuppressive drug is provided to said subject at a sub-optimal dose.

42. The method of claim 41, wherein said immunosuppressive drug is selected from the group consisting of dexamethesone, azathioprine and cyclosporin A.

15 43. The method of any one of claims 16, or 35-37, wherein said anti-inflammatory agent is provided prophylactically to said subject.

44. The method of any one of claims 16, or 35-37, wherein said anti-inflammatory agent is provided therapeutically to said subject.

20 45. A method of suppressing the metastasis of a hematopoietic tumor cell, said cell requiring a functional member of the LFA-1 family for migration, which method comprises providing to a patient in need of such treatment an amount of an anti-inflammatory agent sufficient to suppress said metastasis; wherein said anti-inflammatory agent being selected from the group consisting of: an antibody capable of binding to ICAM-1; a fragment of an antibody, said fragment being capable of
25 binding to ICAM-1; ICAM-1; a functional derivative of ICAM-1; and a non-immunoglobulin antagonist of ICAM-1.

46. The method of claim 45, wherein said non-immunoglobulin antagonist of ICAM-1 is a non-immunoglobulin antagonist of ICAM-1 other than LFA-1.

5 47. The method of claim 45, wherein said anti-inflammatory agent is an antibody capable of ~~binding~~ to ICAM-1.

48. The method of claim 47, wherein said antibody is a monoclonal antibody.

49. The method of claim 48, wherein said monoclonal antibody is the monoclonal antibody R6-5-D6.

10 50. The method of claim 45, wherein said anti-inflammatory agent is a fragment of an ~~antibody~~, said fragment being capable of binding to ICAM-1.

51. The method of claim 50, wherein said fragment is a fragment of the antibody R6-5-D6.

15 ✓ 52. A method of suppressing the growth of an ICAM-1-expressing tumor cell which comprises providing to a patient in need of such treatment an amount of a toxin sufficient to suppress said growth, said toxin being selected from the group consisting of a toxin-derivatized antibody capable of binding to ICAM-1; a toxin-derivatized fragment of
20 an antibody, said fragment being capable of binding to ICAM-1; a toxin-derivatized member of the LFA-1 family of molecules; and a toxin-derivatized functional derivative of a member of the LFA-1 family of molecules.

25 ✓ 53. A method of suppressing the growth of an LFA-1-expressing tumor cell which comprises providing to a patient in need of such treatment an amount of a toxin sufficient to suppress said growth, said toxin

being selected from the group consisting of a toxin-derivatized ICAM-1; and a toxin-derivatized functional derivative of ICAM-1.

54. A method of diagnosing the presence and location of inflammation resulting from a response of the specific defense system in a mammalian subject suspected of having said inflammation which comprises:

(a) administering to said subject a composition containing a detectably labeled binding ligand capable of identifying a cell which expresses ICAM-1, and

(b) detecting said binding ligand.

55. A method of diagnosing the presence and location of inflammation resulting from a response of the specific defense system in a mammalian subject suspected of having said inflammation which comprises:

(a) incubating a sample of tissue of said subject with a composition containing a detectably labeled binding ligand capable of identifying a cell which expresses ICAM-1, and

(b) detecting said binding ligand.

56. The method of any one of claims 54 or 55 wherein said binding ligand is bound in said sample of ~~said tissue~~.

57. The method of any one of claims 54 or 55 wherein said binding ligand is capable of binding to ICAM-1, said ligand being selected from the group consisting of an antibody and a fragment of an antibody.

58. The method of claim 57, wherein said antibody is a monoclonal antibody.

59. The method of claim 58, wherein said monoclonal antibody is the monoclonal antibody R6-5-D6.

60. The method of any one of claims 54 or 55 wherein said binding ligand is a nucleic acid molecule capable of binding to a molecule selected from the group consisting of a DNA sequence of ICAM-1, and an mRNA sequence of a gene for ICAM-1.

5 61. The method of claim 60 wherein said nucleic acid molecule encodes at least one polypeptide selected from the group consisting of:

- (a) -V-T-C-S-T-S-C-D-Q-P-K;
- (b) -X-G-S-V-L-V-T-C-S-T-S-C-D-Q-P-K;
- (c) -L-L-G-I-E-T-P-L;
- 10 (d) -F-L-T-V-Y-X-T;
- (e) -V-E-L-A-P-L-P;
- (f) -E-L-D-L-R-P-Q-G-L-E-L-F-E;
- (g) -L-N-P-T-V-T-Y-G-X-D-S-F-S-A-K;
- (h) -S-F-P-A-P-N-V;
- 15 (i) -L-R-G-E-K-E-L;
- (j) -R-G-E-K-E-L-K-R-E-P;
- (k) -L-R-G-E-K-E-L-K-R-E-P-A-V-G-E-P-A-E;
- (l) -P-R-G-G-S;
- (m) -P-G-N-N-R-K;
- 20 (n) -Q-E-D-S-Q-P-M;
- (o) -T-P-E-R-V-E-L-A-P-L-P-S;
- (p) -R-R-D-H-H-G-A-N-F-S; and
- (q) -D-L-R-P-Q-G-L-E.

✓ 25 62. A method of diagnosing the presence and location of an ICAM-1-expressing tumor cell in a mammalian subject suspected of having such a cell, which comprises:

(a) administering to said subject a composition containing a detectably labeled binding ligand capable of binding to ICAM-1, said ligand being selected from the group consisting of an antibody and a
30 fragment of an antibody, said fragment being capable of binding to ICAM-1, and

(b) detecting said binding ligand.

✓ 35 63. A method of diagnosing the presence and location of inflammation resulting from a response of the specific defense system in a mammalian subject suspected of having said inflammation which comprises:

- (a) incubating a sample of tissue of said subject with a composition containing a detectably labeled binding ligand capable of identifying a cell which expresses ICAM-1, and
- (b) detecting said binding ligand.

5 64. The method of any one of claims 62 or 63, wherein said binding ligand is bound to ICAM-1 present in said sample of tissue.

10 65. The binding ligand of any one of claims 62 or 63, wherein said binding ligand is selected from the group consisting of: a monoclonal antibody capable of binding to ICAM-1; and a fragment of said monoclonal antibody, said fragment being capable of binding to ICAM-1.

66. The binding ligand of claim 65, wherein said monoclonal antibody is the monoclonal antibody R6-5-D6.

15 ✓ 67. A method of diagnosing the presence and location of a tumor cell which expresses a member of the LFA-1 family of molecules in a subject suspected of having such a cell, which comprises:

- (a) administering to said subject a composition containing a detectably labeled binding ligand capable of binding to a member of the LFA-1 family of molecules, said ligand being selected from the group consisting of ICAM-1 and a functional derivative of ICAM-1 and
- 20 (b) detecting said binding ligand.

25 ✓ 68. A method of diagnosing the presence and location of a tumor cell which expresses a member of the LFA-1 family of molecules in a subject suspected of having such a cell, which comprises:

- (a) incubating a sample of tissue of said subject in the presence of a composition containing a detectably labeled binding ligand capable of binding to a member of the LFA-1 family of molecules, said ligand being selected from the group consisting of ICAM-1 and a functional derivative of ICAM-1 and

(b) detecting said binding ligand which is bound to a member of the LFA-1 family of molecules present in said sample of tissue.

✓ 69. A pharmaceutical composition comprising:

- 5 (a) an anti-inflammatory agent selected from the group consisting of: an antibody capable of binding to ICAM-1; a fragment of an antibody, said fragment being capable of binding to ICAM-1; ICAM-1; a functional derivative of ICAM-1; and a non-immunoglobulin antagonist of ICAM-1, and
- 10 (b) at least one immunosuppressive agent selected from the group consisting of: dexamethesone, azathioprine and cyclosporin A.

70. The pharmaceutical composition of claim 69 which contains one immunosuppressive agent, said immunosuppressive agent being selected from the group consisting of: dexamethesone, azathioprine and cyclosporin A.